H42 Cont...

obtained. There were no differences between the testes and the pGRN121 sequences from in this region.

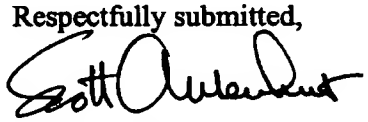
REMARKS

Pursuant to the Notice of Draft Person's Patent Drawing Review dated March 28, 2002, Applicants hereby submit new drawings that incorporate the new figure numbers as requested.

The amendments to the specification are generally simply to amend the figure references for those figures in which the figure number was amended in accordance with the request of the draftsperson, and to correct the form of certain sequence identification numbers. Base numbers have been inserted on page 167, lines 20 and 23; these numbers can be determined from the coding and intron regions illustrated in Figures 21C and 21E and the sequence listed in SEQ ID NO: 6. No new matter has been added.

Entry of this amendment is respectfully urged since it merely cures formal defects in the specification and does not touch the merits.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The paragraph beginning at line 1 of page 8 has been amended as follows:

The invention also provides a method for treatment of a condition associated with an elevated level of telomerase activity within a cell, comprising introducing into said cell a therapeutically effective amount of an inhibitor of said telomerase activity, wherein said inhibitor is an hTRT polypeptide or an hTRT polynucleotide. In one embodiment the inhibitor is a polypeptide comprising the sequence of ~~SEQ. ID. NO: 2 or 4~~ SEQ ID NO: 2 or 4, or a subsequence thereof. In additional embodiments the polypeptide inhibits a TRT activity, such as binding of endogenous TRT to telomerase RNA.

The paragraph beginning at line 10 of page 9 has been amended as follows:

~~**Figure 10 shows**~~ **Figures 10A and 10B** show coexpression *in vitro* of the hTRT and hTR to produce catalytically active human telomerase.

The paragraph beginning at line 19 of page 9 has been amended as follows:

~~**Figure 13 (SEQ. ID. NO: 109) shows**~~ **Figures 13A and 13B** (SEQ ID NO: 109) show the sequence of the DNA encoding the *Euplotes* 123 kDa telomerase protein subunit.

The paragraph beginning at line 23 of page 9 has been amended as follows:

~~**Figure 15 (SEQ. ID NOS: 111-112) shows**~~ **Figures 15A-15F** (SEQ ID NOS: 111-112) show the DNA and amino acid sequences of the *S. pombe* telomerase catalytic subunit.

The paragraph beginning at line 1 of page 10 has been amended as follows:

~~**Figure 20 shows**~~ **Figures 20A-20E** show the sequence of a nucleic acid encoding a Δ182 variant polypeptide **(SEQ ID NO: 4)**.

The paragraph beginning at line 3 of page 10 has been amended as follows:

~~**Figure 21 shows**~~ **Figures 21A-21E** show the sequence from an hTRT genomic clone **(SEQ ID NO: 6)**.

The paragraph beginning at line 26 of page 11 has been amended as follows:

As described in detail in the above-referenced priority documents, TRT was initially characterized following purification of telomerase from the ciliate *Euplotes aediculatus*. Extensive purification of *E. aediculatus* telomerase, using RNA-affinity chromatography and other methods, yielded the protein "p123". Surprisingly, p123 is unrelated to proteins previously believed to constitute the protein subunits of the telomerase holoenzyme (i.e., the p80 and p95 proteins of *Tetrahymena thermophila*). Analysis of the p123 DNA and protein sequences (Genbank Accession No. U95964; ~~**Figures 13 and 14**~~ **Figures 13A, 13B and 14**) revealed reverse transcriptase (RT) motifs consistent with the role of p123 as the catalytic subunit of telomerase (see, e.g., **Figure 1**). Moreover, p123 is related to a *S. cerevisiae* (yeast) protein, Est2p, which was known to play a role in maintenance of telomeres in *S. cerevisiae* (Genbank Accession No. S5396), but prior to the present invention was not recognized as encoding a telomerase catalytic subunit protein (see, e.g., Lendvay et al., 1996, *Genetics*, 144:1399).

The paragraph beginning at line 28 of page 12 has been amended as follows:

The *Euplotes* p123, *S. pombe* trt1, and *S. cerevisiae* Est2p sequences of the invention were used in a search of a computerized database of human expressed sequence tags (ESTs) using the program BLAST (Altschul et al, 1990, *J. Mol. Biol.* 215:403). Searching this database with the Est2p sequence did not indicate a match, but searching with p123 and trt1 sequences identified a human EST (Genbank accession no. AA281296), as described in **Example 1**, putatively encoding a homologous protein. Complete sequencing of the cDNA clone containing the EST (hereinafter, "clone 712562"; see ~~**SEQ. ID. NO: 3**~~ **SEQ ID NO: 3**) showed that seven RT motifs were present. However, this clone could not encode a contiguous human TRT because motifs B', C, D, and E were contained in a different open reading frame (ORF) than the more NH_2-terminal motifs. In addition, the distance between motifs A and B' was substantially shorter than that of the three previously characterized TRTs. (Clone 712562 was obtained from the I.M.A.G.E. Consortium; Lennon et al., 1996, *Genomics* 33:151).

The paragraph beginning at line 12 of page 13 has been amended as follows:

A cDNA clone, pGRN121, encoding a functional hTRT (~~SEQ. ID. NO: 1~~ SEQ ID NO: 1) was isolated from a cDNA library derived from the human 293 cell line as described in **Example 1**. Comparing clone 712562 with pGRN121 showed that clone 712562 has a 182 base pair (**SEQ ID NO: 9**) deletion between motifs A and B'. The additional 182 base pairs present in pGRN121 places all of the TRT motifs in a single open reading frame, and increases the spacing between the motif A and motif B' regions to a distance consistent with the other known TRTs. As is described *infra* in the Examples (e.g., **Example 7**), ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1 encodes a catalytically active telomerase protein having the sequence of **SEQ ID NO: 2**. The polypeptide of **SEQ ID NO: 2** has 1132 residues and a calculated molecular weight of about 127 kilodaltons (kD).

The paragraph beginning at line 22 of page 13 has been amended as follows:

As is discussed *infra*, ~~*and*~~ and described in **Example 9**, *infra*, TRT cDNAs possessing the 182 basepair deletion characteristic of the clone 712562 are detected following reverse transcription of RNA from telomerase-positive cells (e.g., testis and 293 cells). hTRT RNAs lacking this 182 base pair sequence are referred to generally as "Δ182 variants" and may represent one, two, or several species. Although the hTRT variants lacking the 182 basepair sequence found in the pGRN121 cDNA (~~SEQ ID NO. 1~~ SEQ ID NO: 1) are unlikely to encode a fully active telomerase catalytic enzyme, they may play a role in telomerase regulation, as discussed *infra*, and/or have partial telomerase activity, such as telomere binding or hTR binding activity, as discussed *infra*.

The paragraph beginning at line 3 of page 21 has been amended as follows:

The present invention provides isolated and recombinant nucleic acids having a sequence of a polynucleotide encoding a telomerase catalytic subunit protein (TRT), such as a recombinant TRT gene from *Euplotes, Tetrahymena, S. pombe* or humans. Exemplary polynucleotides are provided in ~~**Figure 13**~~ **Figures 13A and 13B** (*Euplotes*); ~~**Figure 15**~~ **Figures 15A-15F** (*S. pombe*) and **Figure 16** (human, GenBank Accession No. AF015950). The present invention provides sense and anti-sense polynucleotides having a TRT gene sequence, including probes, primers, TRT-protein-encoding polynucleotides, and the like.

The paragraph beginning at line 14 of page 21 has been amended as follows:

In one aspect, the invention provides a polynucleotide having a sequence or subsequence of a human TRT gene or RNA. In one embodiment, the polynucleotide of the invention has a sequence of ~~SEQ. ID NO: 1~~ SEQ ID NO: 1, or a subsequence thereof. In another embodiment, the polynucleotide has a sequence of ~~SEQ. ID NO: 3~~ SEQ ID NO: 3 **(Figure 18)**, ~~SEQ. ID. NO: 4~~ SEQ ID NO: 4 ~~**(Figure 20)**~~ **(Figures 20A-20E)**, or subsequences thereof. The invention also provides polynucleotides with substantial sequence identity to the hTRT nucleic acid sequences disclosed herein, e.g., ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1 and any others disclosed (e.g., ~~SEQ. ID. NOS: 4, 6 [Figure 21]~~ SEQ ID NOS: 4, 6 [Figures 21A-21E], and 7 **[Figure 22]**). Thus, the invention provides naturally occurring alleles of human TRT genes and variant polynucleotide sequences having one or more nucleotide deletions, insertions or substitutions relative to an hTRT nucleic acid sequence disclosed herein. As described *infra*, variant nucleic acids may be produced using the recombinant or synthetic methods described below or by other means.

The paragraph beginning at line 27 of page 21 has been amended as follows:

The invention also provides isolated and recombinant polynucleotides having a sequence from a flanking region of a human TRT gene. Such polynucleotides include those derived from genomic sequences of untranslated regions of the hTRT mRNA. An exemplary genomic sequence is ~~SEQ. ID. NO: 6~~ SEQ ID NO: 6. As described in **Example 4**, ~~SEQ. ID. NO. 6~~ SEQ ID NO: 6 was obtained by sequencing a clone, λGΦ5 isolated from a human genomic library. Lambda GΦ5 contains a 15 kilobasepair (kbp) insert including approximately 13,000 bases 5' to the hTRT coding sequences. This clone contains hTRT promoter sequences and other hTRT gene regulatory sequences (e.g., enhancers).

The paragraph beginning at line 6 of page 22 has been amended as follows:

The invention also provides isolated and recombinant polynucleotides having a sequence from an intronic region of a human TRT gene. An exemplary intronic sequence is ~~SEQ. ID. NO: 7~~ SEQ ID NO: 7 (see **Example 3**). In some embodiments, hTRT introns are included in "minigenes" for improved expression of hTRT proteins in eukaryotic cells.

The paragraph beginning at line 27 of page 22 has been amended as follows:

In particular embodiments, the invention provides hTRT oligo- and polynucleotides that comprise a subsequence of an hTRT nucleic acid disclosed herein (e.g., **SEQ ID NOS: 1, 4, 6, and 7**). The nucleic acids of the invention typically comprise at least about 10, more often at least about 12 or about 15 consecutive bases of the exemplified hTRT polynucleotide. Often, the nucleic acid of the invention will comprise a longer sequence, such as at least about 25, about 50, about 100, about 200, or at least about 500 bases in length, for example when expression of a polypeptide is intended. In some embodiments of the present invention, the hTRT polynucleotide is other than a polynucleotide having the sequence of EST AA281296 (~~**SEQ. ID NO. 8**~~ **SEQ ID NO: 8**).

The paragraph beginning at line 6 of page 23 has been amended as follows:

In still other embodiments, the present invention provides "Δ182 hTRT" polynucleotides having a sequence encoding naturally occurring or non-naturally occurring hTRT polynucleotides such as **SEQ ID NO: 3** or **SEQ ID NO: 4**, which do not contain the 182 basepair sequence (**SEQ ID NO: 9 [Figure 24]**) found in pGRN121 (and also absent in clone 712562). These polynucleotides are of interest, in part, because they encode polypeptides that contain different combinations of TRT motifs than found in the "full-length" hTRT polypeptide (~~**SEQ. ID. NO. 2**~~ **SEQ ID NO: 2**) such as is encoded by pGRN121. As discussed *infra*, it is contemplated that these polypeptides may play a biological role in nature (e.g., in regulation of telomerase expression in cells) and/or find use as therapeutics (e.g., as dominant-negative products that inhibit function of wild-type proteins), or have other roles and uses, e.g. as described herein.

The paragraph beginning at line 17 of page 23 has been amended as follows:

For example, in contrast to the polypeptide encoded by pGRN121, clone 712562 encodes a 259 residue protein with a calculated molecular weight of approximately 30 kD (hereinafter, "712562 hTRT"). The 712562 hTRT polypeptide (~~**SEQ. ID NO: 10**~~ **SEQ ID NO: 10 [Figure 19]**) contains motifs T, 1, 2, and A, but not motifs B', C, D and E. Similarly, a variant hTRT polypeptide with therapeutic and other activities may be expressed from a nucleic acid similar to

the pGRN121 cDNA but lacking the 182 basepairs missing in clone 712562, e.g., having the sequence ~~SEQ. ID. NO.: 4~~ SEQ ID NO: 4. This nucleic acid (hereinafter, "pro90 hTRT"), which may be synthesized using routine synthetic or recombinant methods as described herein, encodes a protein of 807 residues (calculated molecular weight of approximately 90 kD) that shares the same amino terminal sequence as the hTRT protein encoded by ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1, but diverges at the carboxy-terminal region (the first 763 residues are common, the last 44 residues of pro90 hTRT are different than "full-length" hTRT). The pro90 hTRT polypeptide contains motifs T, 1, 2, and A, but not motifs B, C, D, E, and thus may have some, but not all telomerase activities.

The paragraph beginning at line 17 of page 27 has been amended as follows:

As noted *supra*, the present invention provides nucleic acids having flanking (5' or 3') and intronic sequences of the hTRT gene. The nucleic acids are of interest, *inter alia*, because they contain promoter and other regulatory elements involved in hTRT regulation and useful for expression of hTRT and other recombinant proteins or RNA gene products. It will be apparent that, in addition to the nucleic acid sequences provided in ~~SEQ. ID NOS: 6 and 7~~ SEQ ID NOS: 6 and 7, additional hTRT intron and flanking sequences may be readily obtained using routine molecular biological techniques. For example, additional hTRT genomic sequence may be obtained by further sequencing of Lambda clone GΦ5, described *supra* and in **Example 4**. Still other hTRT genomic clones and sequences may be obtained by screening a human genomic library using an hTRT nucleic acid probe having a sequence or subsequence from ~~SEQ. ID. NO. 1~~ SEQ ID NO: 1. Additional clones and sequences (e.g., still further upstream) may be obtained by using labeled sequences or subclones derived from λGΦ5 to probe appropriate libraries. Other useful methods for further characterization of hTRT flanking sequences include those general methods described by Gobinda et al., 1993, *PCR Meth. Applic.* 2:318; Triglia et al., 1988, *Nucleic Acids Res.* 16:8186; Lagerstrom et al., 1991, *PCR Methods Applic.* 1:111; and Parker et al., 1991, *Nucleic Acids Res.* 19:3055.

The paragraph beginning at line 22 of page 28 has been amended as follows:

The 5' untranslated sequences of hTRT or other TRT mRNAs can be determined directly by cloning a "full-length" hTRT or other cDNA using standard methods such as reverse transcription of mRNA, followed by cloning and sequencing the resulting cDNA. Preferred oligo(dT)-primed libraries for screening or amplifying full length cDNAs that have been size-selected to include larger cDNAs may be preferred. Random primed libraries are also suitable and often include a larger proportion of clones that contain the 5' regions of genes. Other well known methods for obtaining 5' RNA sequences, such as the RACE protocol described by Frohman et al., 1988, *Proc. Nat. Acad. Sci USA* 85:8998, may also be used. If desired, the transcription start site of an hTRT or other TRT mRNA can be determined by routine methods using the nucleic acids provided herein (e.g., having a sequence of ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1). One method is S1 nuclease analysis (Ausubel et al., *supra*) using a labeled DNA having a sequence from the 5' region of **SEQ ID NO: 1**.

The paragraph beginning at line 16 of page 38 has been amended as follows:

In one embodiment, the hTRT protein of the invention is a polypeptide having a sequence of ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2 **[Figure 17]**, or a fragment thereof. In another embodiment, the hTRT polypeptide differs from ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2 by internal deletions, insertions, or conservative substitutions of amino acid residues. In a related embodiment, the invention provides hTRT polypeptides with substantial similarity to ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2. The invention further provides hTRT polypeptides that are modified, relative to the amino acid sequence of ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2, in some manner, e.g., truncated, mutated, derivatized, or fused to other sequences (e.g., to form a fusion protein). Moreover, the present invention provides telomerase RNPs comprising an hTRT protein of the invention complexed with a template RNA (e.g., hTR). In other embodiments, one or more telomerase-associated proteins is associated with hTRT protein and/or hTR.

The paragraph beginning at line 28 of page 38 has been amended as follows:

The invention also provides other naturally ~~occuring~~ occurring hTRT species or nonnaturally occuring variants, such as proteins having the sequence of, or substantial similarity

to ~~SEQ. ID NO: 5~~ SEQ ID NO: 5 [~~[Figure 20~~ Figures 20A-20E], ~~SEQ. ID. NO. 10~~ SEQ ID NO: 10 [Figure 19], and fragments, variants, or derivatives thereof.

The paragraph beginning at line 2 of page 39 has been amended as follows:

The invention provides still other hTRT species and variants. One example of an hTRT variant may result from ribosome frameshifting of mRNA encoded by the clone 712562 (~~SEQ. ID. NO: 3~~ SEQ ID NO: 3 [Figure 18]) or the pro90 variant hTRT shown in ~~SEQ. ID. NO: 4 [Figure 20]~~ SEQ ID NO: 4 [Figures 20A-20E] and so result in the synthesis of hTRT polypeptides containing all the TRT motifs (for a general example, see, e.g., Tsuchihashi et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:2516; Craigengen et al., 1987, *Cell* 50:1; Weiss, 1990, *Cell* 62:117). Ribosome frameshifting can occur when specific mRNA sequences or secondary structures cause the ribosome to "stall" and jump one nucleotide forwards or back in the sequence. Thus, a ribosome frameshift event on the 712562 mRNA could cause the synthesis of an approximately 523 amino acid residue polypeptide. A ribosome frameshift event on the pro90 sequence could result in a protein with approximately 1071 residues. It will be appreciated that proteins resulting from ribosome frameshifting can also be expressed by synthetic or recombinant techniques provided by the invention.

The paragraph beginning at line 3 of page 45 has been amended as follows:

In an alternative embodiment, the hTRT protein is expressed in a cell (e.g., a telomerase negative cell in which hTR is expressed) as a fusion protein (see *infra*) having a label or an "epitope tag" to aid in purification. In one embodiment, the RNP is recovered from the cell using an antibody that specifically recognizes the tag. Preferred tags are typically short or small and may include a cleavage site or other property that allows the tag to be removed from the hTRT polypeptide. Examples of suitable tags include the Xpress epitope (Invitrogen, Inc., San Diego CA), and other moieties that can be specifically bound by an antibody or nucleic acid or other equivalent method such as those described in **Example 6**. Alternative tags include those encoded by sequences inserted, e.g., into ~~SEQ. ID NO: 1~~ SEQ ID NO: 1 upstream of the ATG codon that initiates translation of the protein of ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2, which may include insertion of a (new) methionine initiation codon into the upstream sequence.

The paragraph beginning at line 8 of page 66 has been amended as follows:

In a related aspect, the present invention provides antibodies that are specifically immunoreactive with hTRT, including polyclonal and monoclonal antibodies, antibody fragments, single chain antibodies, human and chimeric antibodies, including antibodies or antibody fragments fused to phage coat or cell surface proteins, and others known in the art and described herein. The antibodies of the invention can specifically recognize and bind polypeptides that have an amino acid sequence that is substantially identical to the amino acid sequence of ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2, or an immunogenic fragment thereof or epitope on the protein defined thereby. The antibodies of the invention can exhibit a specific binding affinity for hTRT of at least about 10^7, 10^8, 10^9, or 10^{10} M^{-1}, and may be polyclonal, monoclonal, recombinant or otherwise produced. The invention also provides anti-hTRT antibodies that recognize an hTRT conformational epitope (e.g., an epitope on the surface of the hTRT protein or a telomerase RNP). Likely conformational epitopes can be identified, if desired, by computer-assisted analysis of the hTRT protein sequence, comparison to the conformation of related reverse transcriptases such as the p66 subunit of HIV-1 (see, e.g., **Figure 3**), or empirically. Anti-hTRT antibodies that recognize conformational epitopes have utility, *inter alia*, in detection and purification of human telomerase and in the diagnosis and treatment of human disease.

The paragraph beginning at line 26 of page 66 has been amended as follows:

For the production of anti-hTRT antibodies, hosts such as goats, sheep, cows, guinea pigs, rabbits, rats, or mice, may be immunized by injection with hTRT protein or any portion, fragment or oligopeptide thereof which retains immunogenic properties. In selecting hTRT polypeptides for antibody induction, one need not retain biological activity; however, the protein fragment, or oligopeptide must be immunogenic, and preferably antigenic. Immunogenicity can be determined by injecting a polypeptide and adjuvant into an animal (e.g., a rabbit) and assaying for the appearance of antibodies directed against the injected polypeptide (see, e.g., Harlow and Lane, ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, New York (1988) which is incorporated in its entirety and for all purposes, e.g., at Chapter 5). Peptides used to induce specific antibodies typically have an amino acid sequence consisting of at least

five amino acids, preferably at least 8 amino acids, more preferably at least 10 amino acids. Usually they will mimic or have substantial sequence identity to all or a contiguous portion of the amino acid sequence of the protein of ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2. Short stretches of hTRT protein amino acids may be fused with those of another protein, such as keyhole limpet hemocyanin, and an anti-hTRT antibody produced against the chimeric molecule. Depending on the host species, various adjuvants may be used to increase immunological response.

The paragraph beginning at line 30 of page 82 has been amended as follows:

In one aspect of the invention, a telomerase modulatory polypeptide that increases telomerase activity in a cell is provided. In one embodiment, the polypeptide is a catalytically active hTRT polypeptide capable of directing the synthesis (in conjunction with an RNA template such as hTR) of human telomeric DNA. This activity can be measured, as discussed above, e.g., using a telomerase activity assay such as a TRAP assay. In one embodiment, the polypeptide is a full-length hTRT protein, having a sequence of, or substantially identical to, the sequence of 1132 residues of ~~SEQ. ID. No: 2~~ SEQ ID NO: 2. In another embodiment, the polypeptide is a variant of the hTRT protein of ~~SEQ. ID. No: 2~~ SEQ ID NO: 2, such as a fusion polypeptide, derivatized polypeptide, truncated polypeptide, conservatively substituted polypeptide, or the like. A fusion or derivatized protein may include a targeting moiety that increases the ability of the polypeptide to traverse a cell membrane or causes the polypeptide to be preferentially delivered to a specified cell type (e.g., liver cells or tumor cells) or cell compartment (e.g., nuclear compartment). Examples of targeting moieties include lipid tails, amino acid sequences such as antennopedia peptide (see USSN 08/838,545, filed 9 April 1997) or a nuclear localization signal (NLS; e.g., *Xenopus* nucleoplasmin Robbins et al., 1991, *Cell* 64:615). Naturally occurring hTRT protein (e.g., having a sequence of, or substantially identical to, ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2) acts in the cell nucleus. Thus, it is likely that one or more subsequences of ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2, such as residues 193-196 (PRRR) and residues 235-240 (PKRPRR) act as a nuclear localization signal. The small regions are likely NLSs based on the observation that many NLSs comprise a 4 residue pattern composed of basic amino acids (K or R), or composed of three basic amino acids (K or R) and H or P; a pattern

starting with P and followed within 3 residues by a basic segment containing 3 K or R residues out of 4 residues. See Nakai et al., 1992, *Genomics* 14:897. Deletion of one or both of these sequences and/or additional localization sequences is expected to interfere with hTRT transport to the nucleus and/or increase hTRT turnover, and is useful for preventing access of telomerase to its nuclear substrates and decreasing proliferative potential. Moreover, a variant hTRT polypeptide lacking NLS may assemble into an RNP that will not be able to maintain telomere length, because the resulting enzyme cannot enter the nucleus.

The paragraph beginning at line 30 of page 83 has been amended as follows:

The hTRT polypeptides of the invention will typically be associated in the target cell with a telomerase RNA, such as hTR, when they are used to increase telomerase activity in a cell. In one embodiment, an introduced hTRT polypeptide associates with an endogenous hTR to form a catalytically active RNP (e.g., an RNP comprising the hTR and a full-length polypeptide having a sequence of ~~SEQ. ID. NO. 2~~ SEQ ID NO: 2). The RNP so formed may also associate with other, e.g., telomerase-associated, proteins. In other embodiments, telomerase RNP (containing hTRT protein, hTR and optionally other components) is introduced as a complex to the target cell.

The paragraph beginning at line 25 of page 86 has been amended as follows:

In one embodiment, the antisense sequence is complementary to relatively accessible sequences of the hTRT mRNA (e.g., relatively devoid of secondary structure). This can be determined by analyzing predicted RNA secondary structures using, for example, the MFOLD program (Genetics Computer Group, Madison WI) and testing *in vitro* or *in vivo* as is known in the art. Examples of oligonucleotides that may be tested in cells for antisense suppression of hTRT function are those capable of hybridizing to (i.e., substantially complementary to) the following positions from ~~SEQ. ID. NO:1~~ SEQ ID NO: 1: 40-60; 260-280; 500-520; 770-790; 885-905; 1000-1020 ; 1300-1320; 1520-1540; 2110-2130; 2295-2315; 2450-2470; 2670-2690; 3080-3110; 3140-3160; and 3690-3710. Another useful method for identifying effective antisense compositions uses combinatorial arrays of oligonucleotides (see, e.g., Milner et al., 1997, *Nature Biotechnology* 15:537).

The paragraph beginning at line 12 of page 87 has been amended as follows:

The antisense nucleic acids (DNA, RNA, modified, analogues, and the like) can be made using any suitable method for producing a nucleic acid, such as the methods disclosed herein. In one embodiment, for example, antisense RNA molecules of the invention may be prepared by *de novo* chemical synthesis or by cloning. For example, an antisense RNA that hybridizes to hTRT mRNA can be made by inserting (ligating) an hTRT DNA sequence (e.g., ~~Seq. ID No. 1~~ SEQ ID NO: 1, or fragment thereof) in reverse orientation operably linked to a promoter in a vector (e.g., plasmid). Provided that the promoter and, preferably termination and polyadenylation signals, are properly positioned, the strand of the inserted sequence corresponding to the noncoding strand will be transcribed and act as an antisense oligonucleotide of the invention.

The paragraph beginning at line 22 of page 90 has been amended as follows:

Gene therapy refers to the introduction of an otherwise exogenous polynucleotide which produces a medically useful phenotypic effect upon the (typically) mammalian cell(s) into which it is transferred. In one aspect, the present invention provides gene therapy methods and compositions for treatment of telomerase-associated conditions. In illustrative embodiments, gene therapy involves introducing into a cell a vector that expresses an hTRT gene product (such as an hTRT protein substantially similar to the hTRT polypeptide having a sequence of ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2, e.g., to increase telomerase activity, or an inhibitory hTRT polypeptide to reduce activity), expresses a nucleic acid having an hTRT gene or mRNA sequence (such as an antisense RNA, e.g., to reduce telomerase activity), expresses a polypeptide or polynucleotide that otherwise affects expression of hTRT gene products (e.g., a ribozyme directed to hTRT mRNA to reduce telomerase activity), or replaces or disrupts an endogenous hTRT sequence (e.g., gene replacement and "gene knockout," respectively). Numerous other embodiments will be evident to one of skill upon review of the disclosure herein. In one embodiment, a vector encoding hTR is also introduced. In another embodiment, vectors encoding telomerase-associated proteins are also introduced with or without a vector for hTR.

The paragraph beginning at line 8 of page 92 has been amended as follows:

As noted, the present invention also provides methods and reagents for gene replacement therapy (i.e., replacement by homologous recombination of an endogenous hTRT gene with a recombinant gene). Vectors specifically designed for integration by homologous recombination may be used. Important factors for optimizing homologous recombination include the degree of sequence identity and length of homology to chromosomal sequences. The specific sequence mediating homologous recombination is also important, since integration occurs much more easily in transcriptionally active DNA. Methods and materials for constructing homologous targeting constructs are described by e.g., Mansour et al., 1988, *Nature* 336: 348; Bradley et al., 1992, *Bio/Technology* 10: 534. See also, U.S. Patent Nos. 5,627,059; 5,487,992; 5,631,153; and 5,464,764. In one embodiment, gene replacement therapy involves altering or replacing all or a portion of the regulatory sequences controlling expression of the hTRT gene that is to be regulated. For example, the hTRT promoter sequences (e.g., such as are found in ~~SEQ. ID. NO. 6~~ SEQ ID NO: 6) may be disrupted (to decrease hTRT expression or to abolish a transcriptional control site) or an exogenous promoter (e.g., to increase hTRT expression) substituted.

The paragraph beginning at line 14 of page 100 has been amended as follows:

In one embodiment, a polynucleotide comprising a sequence encoding a polypeptide of ~~SEQ. ID. NO: 2~~ SEQ ID NO: 2, which sequence is operably linked to a promoter (e.g., a constitutively expressed promoter, e.g., a sequence of ~~SEQ. ID. NO: 6~~ SEQ ID NO: 6), is introduced into the cell. In one embodiment the polynucleotide comprises a sequence of ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1. Preferably the polynucleotide includes polyadenylation and termination signals. In other embodiments, additional elements such as enhancers or others discussed *supra* are included. In an alternative embodiment, the polynucleotide does not include a promoter sequence, such sequence being provided by the target cell endogenous genome following integration (e.g., recombination, e.g., homologous recombination) of the introduced polynucleotide. The polynucleotide may be introduced into the target cell by any method, including any method disclosed herein, such as lipofection, electroporation, virosomes, liposomes, immunoliposomes, polycation:nucleic acid conjugates, naked DNA).

The paragraph beginning at line 6 of page 131 has been amended as follows:

One example of an hTRT variant gene product that may be detected is an hTRT RNA such as a product (~~SEQ. ID. NO: 4~~ SEQ ID NO: 4) described *supra* and in **Example 9**. The biological function, if any, of the Δ182 variant(s) is not known; however, the truncated hTRT protein putatively encoded by the variant may be involved in regulation of telomerase activity, e.g., by assembling a non-functional telomerase RNP that titrates telomerase components. Alternatively, negative regulation of telomerase activity could be accomplished by directing hTRT pre-mRNA (nascent mRNA) processing in a manner leading to elimination of the mRNA and reducing hTRT mRNA levels. For these and other reasons, the ability to detect Δ182 variants is useful. In addition, it will sometimes be desirable, in samples in which two species of hTRT RNA are present (such as a Δ182 hTRT RNA and hTRT encoding the full-length hTRT protein) to compare their relative and/or absolute abundance.

The paragraph beginning at line 29 of page 131 has been amended as follows:

Another suitable method entails PCR amplification (or the equivalent) using three primers. Analogous to the semi-competitive quantitative PCR method described in greater detail *supra*, one primer is specific to each of the hTRT RNA species (e.g., as illustrated in **Table 4**) and one primer is complementary to both species (e.g., TCP1.25 (2270-2288)). An example of a primer specific to ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1 is one that anneals within the 182 nucleotide sequence (i.e., nucleotides 2345 to 2526 of ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1), e.g., TCP1.73 (2465-2445). For example, a primer specific to ~~SEQ. ID. No. 4~~ SEQ ID NO: 4 (a Δ182 variant) is one that anneals at nucleotides 2358 to 2339 of ~~SEQ. ID. NO: 4~~ SEQ ID NO: 4 (i.e., the site corresponding to the 182 nucleotide insertion in ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1). The absolute abundance of the Δ182 hTRT mRNA species or its relative abundance compared to the species encoding the full-length hTRT protein can be analyzed for correlation to cell state (e.g., capacity for indefinite proliferation). It will be appreciated that numerous other primers may be selected based on the present disclosure.

The paragraph beginning at line 27 of page 165 has been amended as follows:

As shown below (SEQ ID NO: 313), the 104-base intronic sequence (**SEQ ID NO: 7**) is inserted in the hTRT mRNA (shown in bold) at the junction corresponding to bases 274 and 275 of ~~**SEQ. ID. NO: 1.**~~ **SEQ ID NO: 1.**

The paragraph beginning at line 8 of page 166 has been amended as follows:

This intron contains motifs characteristic of a topoisomerase II cleavage site and a NFκB binding site (see ~~**Figure 21**~~ **Figures 21A-21E**). These motifs are of interest, in part, because expression of topoisomerase II is up regulated in most tumors. It functions to relax DNA by cutting and rewinding the DNA, thus increasing expression of particular genes. Inhibitors of topoisomerase II have been shown to work as anti-tumor agents. In the case of NFκB, this transcription factor may play a role in regulation of telomerase during terminal differentiation, NFκB may play a role in early repression of telomerase during development and so is another target for therapeutic intervention to regulate telomerase activity in cells.

The paragraph beginning at line 28 of page 166 has been amended as follows:

The genomic library was divided into pools of 150,000 phage each, and each pool screened by nested PCR (outer primer pair TCP1.52 & TCP1.57; inner pair TCP1.49 & TCP1.50, see **Table 1**). These primer pairs span a putative intron (see **Example 3,** *supra*) in the genomic DNA of hTRT and ensured the PCR product was derived from a genomic source and not from contamination by the hTRT cDNA clone. Positive pools were further subdivided until a pool of 2000 phage was obtained. This pool was plated at low density and screened via hybridization with a DNA fragment encompassing basepairs 1552-2108 of ~~**SEQ. ID. NO. 1**~~ **SEQ ID NO: 1** (restriction sites SphI and EcoRV, respectively).

The paragraph beginning at line 11 of page 167 has been amended as follows:

Phage GΦ5 was mapped by restriction enzyme digestion and DNA sequencing. The resulting map is shown in **Figure 7**. The phage DNA was digested with *Nco*I and the fragments cloned into pBBS167. The resulting subclones were screened by PCR to identify those containing sequence corresponding to the 5' region of the hTRT cDNA. A subclone (pGRN140) containing a 9 kb *Nco*I fragment (with hTRT gene sequence and 4-5 kb of lambda vector

sequence) was partially sequenced to determine the orientation of the insert. pGRN 140 was digested using *SalI* to remove lambda vector sequences, resulting in pGRN144. pGRN144 was then sequenced. The sequence is provided in ~~Seq. ID. NO: 6~~ SEQ ID NO: 6. The 5' end of the hTRT mRNA corresponds to ~~base~~ base 2258 of ~~Seq. ID. NO: 6~~ SEQ ID NO: 6. As indicated in **Figure 7**, two Alu sequence elements are located 1700 base pairs upstream of the hTRT cDNA 5' end and provide a likely upstream limit to the promoter region of hTRT. The sequence also reveals an intron positioned at ~~bases~~ base 4173 ~~Seq. ID. NO: 6~~ SEQ ID NO: 6, 3' to the intron described in **Example 3**, *supra.*

The paragraph beginning at line 6 of page 168 has been amended as follows:

The human BAC clone (326 E 20) was obtained with a hybridization screen of a BAC human genomic library using an 1143 bp Sph1/Xmn1 fragment of ~~SEQ. ID. NO: 1~~ SEQ ID NO: 1 (bases 1552-2695) that encompasses the RT motif region. The clone is believed to include the 5' end. The hTRT genomic clones in this example are believed to encompass the entire hTRT gene.

The paragraph beginning at line 2 of page 187 has been amended as follows:

The results of the reconstitution are shown in ~~Figure 10~~ Figures 10A and 10B. For each transcription/translation reaction there are 3 lanes: The first 2 lanes are duplicate assays and the third lane is a heat denatured (95°C, 5 min) sample to rule out PCR generated artifacts.

The paragraph beginning at line 5 of page 187 has been amended as follows:

As shown in ~~Figure 10~~ Figures 10A and 10B, reticulocyte lysate alone has no detectable telomerase activity (lane 6). Similarly, no detectable activity is observed when either hTR alone (lane 1) or full length hTRT gene (lane 4) are added to the lysate. When both components are added (lane 2), telomerase activity is generated as demonstrated by the characteristic repeat ladder pattern. When the carboxy-terminal region of the hTRT gene is removed by digestion of the vector with *NcoI* ("truncated hTRT") telomerase activity is abolished (lane 3). Lane 5 shows that translation of the truncated hTRT also did not generate telomerase activity. Lane

"R8" shows a positive control (TSR8 quantitation standard (SEQ ID NO: 329) (5'-ATTCCGTCGAGCAGAGTTAG[GGTTAG]7-3')).

The paragraph beginning at line 7 of page 190 has been amended as follows:

The testis mRNA sequence corresponding to bases 27 to 3553 of the pGRN121 insert sequence (~~SEQ. ID. NO: 1~~ SEQ ID NO: 1), and containing the entire hTRT ORF (bases 56 to 3451) was obtained. There were no differences between the testes and the pGRN121 sequences from in this region.